

WHAT IS CLAIMED IS:

1. A method of quantifying analyte polynucleotides present in a test sample, comprising the steps of:
  - obtaining a test sample that contains an unknown amount of an analyte polynucleotide;
  - combining a predetermined amount of said test sample with a predetermined amount of a pseudo target;
  - co-amplifying in a polynucleotide amplification reaction the pseudo target and any of the analyte polynucleotide contained in said test sample to produce a collection of amplification products, said collection including both an analyte amplicon if said test sample contained the analyte polynucleotide and a pseudo target amplicon; and
  - quantifying the analyte amplicon without reference to the amount of pseudo target amplicon, whereby the quantity of analyte amplicon is related in a manner that is dose-dependent on the unknown amount of the analyte polynucleotide contained in said test sample.
2. The method of Claim 1, further comprising a step for detecting the pseudo target amplicon.
3. The method of Claim 1, wherein the step for quantifying comprises hybridizing said collection of amplification products from the co-amplifying step with a labeled probe specific for the analyte amplicon but not the pseudo target amplicon and then detecting any labeled probe that specifically hybridized the analyte amplicon.
4. The method of Claim 3, wherein said polynucleotide amplification reaction in the co-amplifying step is selected from the group consisting of a Transcription Mediated Amplification reaction, a NASBA reaction and a Polymerase Chain Reaction.
5. The method of Claim 4, wherein said polynucleotide amplification reaction is the Transcription Mediated Amplification reaction.
6. The method of either Claim 4 or Claim 5, wherein the obtaining step comprises first collecting a biological specimen and then releasing nucleic acids contained therein to result in said test sample that contains the unknown amount of said analyte polynucleotide.
7. The method of Claim 6, further comprising a step for capturing said analyte polynucleotide onto a solid support prior to said co-amplifying step.

8. The method of Claim 7, wherein the solid support is a bead derivatized with a synthetic polynucleotide.

9. The method of Claim 7, wherein the predetermined amount of said pseudo target in the combining step ranges from between  $1.0 \times 10^3$  and  $2 \times 10^8$  molecules.

10. The method of Claim 9, wherein the predetermined amount of said pseudo target in the combining step ranges from between  $1.0 \times 10^4$  and  $2 \times 10^8$  molecules.

11. The method of Claim 10, wherein the predetermined amount of said pseudo target in the combining step ranges from between  $1.0 \times 10^5$  and  $2 \times 10^8$  molecules.

12. The method of Claim 6, wherein the biological specimen is a blood sample or a plasma sample and wherein said nucleic acids comprise viral nucleic acids.

13. The method of Claim 12, wherein the analyte polynucleotide is released from HIV virions.

14. The method of Claim 6, wherein the predetermined amount of the pseudo target in the combining step is between  $1 \times 10^3$  and  $2 \times 10^8$  molecules.

15. The method of Claim 14, wherein the predetermined amount of the pseudo target in the combining step is between  $1 \times 10^4$  and  $2 \times 10^8$  molecules.

16. The method of Claim 15, wherein the predetermined amount of the pseudo target in the combining step is between  $1 \times 10^5$  and  $2 \times 10^8$  molecules.

17. The method of Claim 5, further comprising a step for isolating said analyte polynucleotide and said pseudo target after the combining step and before the co-amplifying step.

18. The method of Claim 5, wherein the predetermined amount of the pseudo target is between  $1 \times 10^3$  and  $2 \times 10^8$  molecules.

19. The method of Claim 18, wherein the predetermined amount of the pseudo target is between  $1 \times 10^4$  and  $2 \times 10^8$  molecules.

20. The method of Claim 19, wherein the predetermined amount of the pseudo target is between  $1 \times 10^5$  and  $2 \times 10^8$  molecules.

21. The method of Claim 3, wherein said labeled probe is labeled with acridinium ester.

22. The method of Claim 21, wherein the quantifying step comprises measuring by luminometry the probe labeled with acridinium ester that specifically hybridized the analyte amplicon.

23. The method of Claim 6, wherein the analyte polynucleotide is a viral polynucleotide.

24. The method of Claim 1, further comprising a step for consulting a standard curve that relates pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon.

25. The method of Claim 5, further comprising a step for consulting a standard curve that relates pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon.

26. The method of Claim 22, further comprising a step for consulting a standard curve that relates pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon.

27. The method of Claim 5, wherein the Transcription Mediated Amplification reaction employs a paired set of oligonucleotide primers have the sequences of SEQ ID NO:1 and SEQ ID NO:2.

28. The method of Claim 27, wherein the pseudo target has a polynucleotide sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:9.

29. A method for relating pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon, said method comprising the steps of:

obtaining a plurality of control samples, wherein each of the control samples has a different predetermined amount of an analyte polynucleotide;

combining each of said plurality of control samples with a constant predetermined amount of a pseudo target to result in a plurality of mixed control samples;

co-amplifying in a plurality of amplification reactions both the pseudo target and the analyte polynucleotide present in each of said plurality of mixed control samples to produce a collection of amplification products that include a pseudo target amplicon and an analyte amplicon;

quantifying the analyte amplicon for each of said plurality of amplification reactions without reference to the amount of pseudo target amplicon present in said collection of amplification products; and

preparing a standard curve having the different predetermined amounts of said analyte polynucleotide plotted against the quantified amounts of said analyte amplicon

produced in each of said plurality of amplification reactions, thereby relating the pre-amplification amounts of said analyte polynucleotide in said plurality of control samples and the post-amplification amounts of analyte amplicon synthesized in the plurality of amplification reactions.

5           30.     The method of Claim 29, further comprising a step for detecting the pseudo target amplicon.

          31.     The method of Claim 29, wherein said analyte polynucleotide is a viral polynucleotide.

10           32.     The method of Claim 31, wherein the viral polynucleotide is an HIV polynucleotide.

          33.     The method of Claim 29, wherein said constant predetermined amount of said pseudo target is between  $1 \times 10^3$  and  $2 \times 10^8$  molecules.

          34.     The method of Claim 33, wherein said constant predetermined amount of said pseudo target is between  $1 \times 10^4$  and  $2 \times 10^8$  molecules.

          35.     The method of Claim 34, wherein said constant predetermined amount of said pseudo target is between  $1 \times 10^3$  and  $2 \times 10^8$  molecules.

          36.     The method of Claim 29, wherein said plurality of amplification reactions in the co-amplifying step is selected from the group consisting of a plurality of Transcription Mediated Amplification reactions, a plurality of NASBA reactions and a plurality of PCR reactions.

20           37.     The method of Claim 36, wherein said plurality of amplification reactions in the co-amplifying step are a plurality of Transcription Mediated Amplification reactions.

          38.     The method of Claim 36 or Claim 37, wherein the quantifying step comprises hybridizing said collection of amplification products from the co-amplifying step with a labeled probe specific for the analyte amplicon but not the pseudo target amplicon and then  
25     quantitatively detecting any labeled probe that specifically hybridized the analyte amplicon.

          39.     The method of Claim 38, wherein the labeled probe is labeled with acridinium ester.

          40.     The method of Claim 38, further comprising a step for capturing said analyte polynucleotide onto a solid support prior to said co-amplifying step.

30           41.     A method of determining whether a biological sample contains an analyte polynucleotide, comprising the steps of:

obtaining a biological sample to be tested for the presence of the analyte polynucleotide;

combining the biological sample with a pseudo target to result in a mixed sample;

5 isolating nucleic acids from the mixed sample, whereby there is obtained a collection of molecules comprising the pseudo target and any of said analyte polynucleotide present in the biological sample;

conducting a polynucleotide amplification reaction to co-amplify the pseudo target and any of said analyte polynucleotide contained in said collection of molecules to  
10 produce amplification products, whereby pseudo target amplicons are formed, and whereby analyte amplicons are formed if said collection of molecules included said analyte polynucleotide;

detecting in said amplification products any of said analyte amplicons without detecting said pseudo target amplicons; and

determining that the biological sample contains said analyte polynucleotide if said analyte amplicons are detected in the amplification products.

42. The method of Claim 41, wherein the amplification reaction is selected from the group consisting of a Transcription Mediated Amplification reaction, a NASBA reaction and a PCR reaction.

43. The method of Claim 42, wherein the amplification reaction is a Transcription Mediated Amplification reaction.

44. The method of Claim 43, wherein the obtaining step comprises drawing blood.

45. The method of either Claim 42 or Claim 43, wherein the detecting step comprises first hybridizing a labeled polynucleotide probe having binding specificity for the  
25 analyte amplicons and then measuring the extent of specific binding of the labeled polynucleotide probe and the analyte amplicons.

46. The method of Claim 45, wherein the isolating step comprises immobilizing said pseudo target and said analyte polynucleotide to a solid support.

47. The method of Claim 45, wherein the detecting step comprises detecting by  
30 luminometry.

48. The method of Claim 47, wherein the analyte polynucleotide is from HIV virions.

49. The method of Claim 48, wherein the pseudo target has a sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:9.

50. A method of determining whether an analyte polynucleotide is present in a test sample in an amount greater or less than a pre-determined value, comprising the steps of:

5 obtaining a test sample to be analyzed for the presence of said analyte polynucleotide;

combining said test sample with an amount of a pseudo target;

10 co-amplifying in a polynucleotide amplification reaction the pseudo target and any analyte polynucleotide contained in said test sample to produce amplification products that include a pseudo target amplicon and an analyte amplicon, wherein said analyte amplicon is present in an amount that is dose-dependent on the amount of said analyte polynucleotide present in said test sample; and

15 quantitatively detecting said analyte amplicon using a detection system calibrated to have a detection threshold corresponding to a signal strength arising from co-amplification of said amount of said pseudo target and an amount of analyte polynucleotide corresponding to said pre-determined value, wherein detection of a signal above or below said threshold of detection indicates that said analyte polynucleotide is present in said test sample in an amount that is respectively greater or less than said pre-determined value.

20 51. A kit for performing a polynucleotide amplification reaction using an analyte polynucleotide as a template, comprising:

a pseudo target;

at least one pair of oligonucleotide primers for co-amplifying the pseudo target and the analyte polynucleotide;

25 reagents for carrying out the polynucleotide amplification reaction, said reagents including deoxynucleotide triphosphates and a DNA polymerizing enzyme; and

printed instructions with directions for first carrying out the amplification reaction and then detecting only analyte amplicons produced in the amplification reaction.

30 52. The kit of Claim 51, further comprising a labeled probe for detecting any analyte amplicons produced in the amplification reaction.

53. The kit of Claim 51, wherein said reagents further include nucleotide triphosphates and an RNA polymerizing enzyme.

54. The kit of Claim 53, wherein the DNA polymerizing enzyme is a reverse transcriptase.

5 55. The kit of Claim 54, wherein no RNase H additional to that provided by said reverse transcriptase is used.

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